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Lifetime reproductive success and longevity of queens in an annual social insect

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Abstract

Although central to understanding life-history evolution, the relationship between lifetime reproductive success and longevity remains uncertain in many organisms. In social insects, no studies have reported estimates of queens' lifetime reproductive success and longevity within populations, despite the importance of understanding how sociality and associated within-group conflict affect life-history traits. To address this issue, we studied two samples of colonies of the annual bumblebee, *Bombus terrestris audax*, reared from wild-caught queens from a single population. In both samples, queens' lifetime reproductive success, measured as either queens' inclusive fitness or as total biomass of queen-produced sexuals (new queens and males), was significantly positively associated with queen longevity, measured from the day the first worker was produced. We suggest that a positive relationship between reproductive success and longevity was inherited from nonsocial ancestors showing parental care and maintained, at least in part, because the presence of workers buffers queens against extrinsic mortality.

Introduction

Identifying the factors affecting lifetime reproductive success within populations is fundamental to the study of life-history evolution (Clutton-Brock, 1988; Roff, 2002). Across a wide range of taxa, a key determinant of lifetime reproductive success is female longevity, with several life-history traits being likely to influence the directionality of this relationship. For example, the positive relationship between female lifetime reproductive success and longevity in mammals is believed to stem from iteroparity (several breeding events per lifetime) and high average lifespans (Weladji *et al.*, 2006; Pettorelli & Durant, 2007). In insects, a negative relationship between number of eggs laid and female longevity occurs in both iteroparous species (e.g. beetle: Fricke *et al.*, 2006) and semelparous species, i.e. species with a single breeding event per lifetime (e.g. parasitoid Hymenoptera: Jervis *et al.*, 2001; Thorne *et al.*, 2006). Such a negative relationship is likely to stem from a trade-off between fecundity early in life and survivorship late in life (Carey, 2001). Parental care is another life-history trait that in principle affects the relationship between female lifetime reproductive success and longevity. By selecting for greater longevity in order that females can provide more care for offspring (Packer *et al.*, 1998; Bourke, 2007), and thereby simultaneously increasing reproductive success, parental care is likely to generate a positive relationship between the two variables. Although there are very few relevant studies, evidence for this in insects comes from a semelparous solitary bee with provisioning of larval cells, in which females exhibited a positive relationship between number of provisioned cells and longevity

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(Bosch & Vicens, 2006). Sociality is similarly believed to lead to increases in both female lifetime reproductive success and longevity (Bourke, 2007). As many social taxa are also likely to have evolved from ancestors with parental care (Alexander, 1974), one might therefore expect female lifetime reproductive success to rise with longevity in social species. However, in addition, sociality introduces potential conflicts between adult group members that affect longevity (Trivers & Hare, 1976; Emlen, 1995; Bourke, 2007), with unknown net consequences for the relationship between female lifetime reproductive success and longevity.

Social insects represent highly suitable organisms in which to study how sociality affects the relationship between female lifetime reproductive success and longevity because demographic data can be collected with relative ease from large samples of social groups and because widespread within-colony conflict (Ratnieks *et al.*, 2006) creates the opportunity to investigate how such conflict affects life-history strategy. In social insects, studies showing that queens live longer than workers in both annual and perennial species (e.g. Spradbery, 1973; Keller & Genoud, 1997) suggest a positive association between high rates of egg production and longevity. However, the relationship between queens' lifetime reproductive success and longevity remains uncertain because no studies have reported estimates of these parameters within single populations of social insects. This is partly because obtaining estimates of lifetime demographic parameters has proved practicable in only a few perennial species (Thorne *et al.*, 2002; Liebig & Poethke, 2004; Wiernasz *et al.*, 2004; Schrempf *et al.*, 2005). Demographic studies over all or most of the colony cycle are more feasible in annual social insects, e.g. vespine wasps (e.g. Archer, 1981; Martin, 1991, 1995), polistine wasps (e.g. Queller & Strassmann, 1988; Ito & Itioka, 2008) and bumblebees (e.g. Müller & Schmid-Hempel, 1992a; Duchateau *et al.*, 2004). In the present study, we determined the relationship between queens' lifetime reproductive success and their longevity (as queens heading colonies), investigated the influence of within-colony conflict, and determined other demographic variables and relationships, in two samples of colonies reared from wild-caught queens from a population of the annual bumblebee *Bombus terrestris*.

Bombus terrestris forms annual colonies founded by single queens (monogyny) after their emergence from diapause (hibernation) in spring (Goulson, 2003). Queens produce workers initially, then sexuals (new queens and males) that enter a panmictic mating population in which queens mate once and males can mate multiple times (Bourke, 1997; Schmid-Hempel & Schmid-Hempel, 2000; Chapman *et al.*, 2003; Lopez-Vaamonde *et al.*, 2004a; Knight *et al.*, 2005). Workers can produce their own male offspring from haploid eggs, but do so mainly in queenless conditions (Alaux *et al.*, 2004; Lopez-Vaamonde *et al.*, 2004a). At the end of the colony cycle, all individuals die except the young, mated queens of the year, who enter diapause (hibernate) before emergence the following spring. Previous demographic data have come from populations of *B. t. terrestris* from Switzerland (e.g. Shykoff & Schmid-Hempel, 1991; Müller *et al.*, 1992; Shykoff & Müller, 1995; Imhoof & Schmid-Hempel, 1999; Baer & Schmid-Hempel, 2003, 2005; Gerloff & Schmid-Hempel, 2005) and the Netherlands (Duchateau & Velthuis, 1988; Beekman & Van Stratum, 1998; Duchateau *et al.*, 2004). The most extensive demographic studies were conducted by Duchateau & Velthuis (1988) and Duchateau *et al.* (2004) on several cohorts of captive colonies from the Netherlands. In this population, queens initially lay diploid eggs yielding workers and then, at the 'switch point', start to lay haploid eggs yielding males. The switch point is a key event in the *B. terrestris* colony cycle because it marks the transition from investment in growth (worker production) to investment in reproduction (sexual production). However, the switch is not completely sharp, as Duchateau *et al.* (2004) found that the percentage of haploid eggs laid by the queen climbed from 0% to 100% over a period of approximately 10 days. At the 'competition point', queen–worker and worker–worker aggression break out as workers start to lay male eggs (Duchateau *et al.*, 2004). The competition point is another key event because it marks the appearance of overt conflict between queen and workers, which can in some colonies culminate in workers killing the queen (worker matricide: Bourke, 1994). In the study of Duchateau *et al.* (2004), colonies divided bimodally into those with relatively early switch points and male-biased sex allocation and those with relatively late switch points and female-biased sex allocation, but the population sex investment ratio was unbiased (1 : 1). Queens with early switch points had hibernated for longer. Duchateau *et al.* (2004) hypothesized that longer hibernation reduced the body condition and hence the expected longevity of emergent queens, so selecting for queens to concentrate on male production because, given a protandrous mating system (Bourke, 1997), early male production is more profitable than early queen production. However, Duchateau *et al.* (2004) did not investigate the relationship between queens' lifetime reproductive success and longevity, as colonies were terminated 25 days after the competition point so as to exclude from consideration worker-produced males (males take 26 days to develop from egg to adult).

In the present study, our goals were to establish the relationship between queens' lifetime reproductive success and longevity and to investigate other factors that potentially affect this relationship, including within-colony conflict. We therefore investigated the demography of colony growth and production over the entire colony cycle of *B. terrestris*, including the period of male production by queenless workers. We calculated queens' lifetime reproductive success both as queens' inclusive

fitness and as biomass of sexual production. An assumption of our calculation of queens' inclusive fitness, which incorporates fitness returns from both a queen's own sons and her workers' sons (queen's grandsons), is that queen- and worker-produced males do not differ in intrinsic quality. Given that male mass in *B. terrestris* influences male mating success (Duchateau & Mariën, 1995; Amin *et al.*, 2007), we tested this assumption by measuring the masses of queen- and worker-produced males. In Hymenopteran societies, life-history strategy is potentially a function of the evolutionary interests of more than one party. In monogynous societies with reproductive workers, such as *B. terrestris* colonies, these parties are the queen, nonreproductive workers and reproductive workers (Pamilo, 1991; Bourke & Ratnieks, 1999). In the present study, we focus primarily on the queens' perspective because current evidence suggests that in bumblebees the queen largely controls sex allocation, the level of worker male production under queenright conditions (i.e. when the queen is alive) and the reproductive schedule (Müller *et al.*, 1992; Alaux *et al.*, 2004, 2005; Duchateau *et al.*, 2004; Lopez-Vaamonde *et al.*, 2004a). However, through matricide, workers conceivably control, at least in some colonies, the timing of the queen's death and hence the amount of worker male production under queenless conditions (Bourke, 1994). We therefore investigated the role of conflict in the life history of the different parties by determining the consequences for workers' direct reproduction (worker male production) of the queen's death occurring at different times.

Methods

Collection and rearing of bees

We studied two samples of colonies of *B. terrestris audax* Harris, the UK subspecies of *B. terrestris* (Alford, 1975). The first (IoZ sample) was reared in 2003 at the Institute of Zoology, London, and the second (QMUL sample) was reared in 2004 at Queen Mary, University of London. Data on reproduction by nonresident (drifter) workers in the IoZ sample were published by Lopez-Vaamonde *et al.* (2004a) and the results of a learning experiment with workers of the QMUL sample were published by Raine *et al.* (2006). In the IoZ sample, 32 colonies were reared from 122 wild queens captured in two areas in or near London, UK (Regent's Park and Silwood Park, Ascot) in February and March 2003. In the QMUL sample, 26 colonies were reared from 295 queens captured in four areas in or near London (Windsor Great Park, Regent's Park, Hyde Park and South Woodford) between February and April 2004. Full details of rearing methods are provided by Lopez-Vaamonde *et al.* (2004a) and Raine *et al.* (2006) respectively. In brief, colonies were housed in wooden nest-boxes in unlit rooms at 28–29 °C and 60–65% relative humidity. Observations and censuses were performed under red light. In the QMUL sample alone, captured queens were first screened for digestive tract parasites and colonies were reared from unparasitized queens (n = 208). In the IoZ sample alone, nest-boxes were connected, at a mean colony size of 37 workers, by plastic tubes to the exterior. This allowed workers to forage freely in the external environment thereafter, although nests were also supplied with artificial nectar and defrosted fresh pollen (Koppert Biological Systems, Haverhill, UK) until 2 weeks after connection to the exterior or until the colony had reached a size of 60 workers. Workers in the QMUL sample were confined to their nest-boxes or to foraging arenas used for learning trials and so never had access to the external environment. QMUL colonies were fed *ad libitum* with sugar solution (Apiinvert®; E. H. Thorne, Market Rasen, UK, or 50% sucrose solution w/w) and defrosted fresh pollen (Koppert Biological Systems). Colony monitoring continued until the death of the last worker, which occurred in all colonies by 21 August 2003 (IoZ sample) or 19 November 2004 (QMUL sample).

Basic demographic and production data and colony growth schedules

All newly eclosed workers and sexuals were counted in daily inspections (eclosion meaning emergence from pupae) and the sexuals were removed. The date of the competition point (IoZ sample alone) and the date of queen's death or disappearance (both samples) were also recorded. In seven QMUL colonies, the date of the queen's death remained unknown because the queen's corpse was not found until the end of the experiment. In the QMUL sample alone, adult workers from 17 of the colonies producing 60 workers or more were subjected to learning trials in a foraging arena, then removed (Raine *et al.*, 2006). Because relatively few workers were removed (mean ± SD 11.5 ± 1.0%, n = 17 colonies), and removals occurred late in the colony cycle (77 ± 4 days after first worker eclosion), these removals are unlikely to have affected colony growth and demography (Müller & Schmid-Hempel, 1992b). In both samples, we assumed worker, male and queen developmental times of 22, 26 and 30 days respectively (Duchateau & Velthuis, 1988; Bourke & Ratnieks, 2001). Therefore, for each colony, the switch point date was estimated as the date of first male eclosion minus 26 days (colony 11 of the IoZ sample had a single male present from the day of first worker eclosion, which was ignored for the purposes of estimating switch point date) and the date at which the first queen-destined egg was laid was estimated as the date of first queen eclosion minus 30 days.

Predictors of queens' lifetime reproductive success

We estimated queens' lifetime reproductive success as queens' inclusive fitness, estimated from sexuals

produced over the entirety of each queen's lifetime and calculated using the function (e.g. Boomsma & Grafen, 1991): quantity of progeny × regression relatedness with progeny × progeny's relative sex-specific reproductive value × progeny's mean relative mating success. For each colony, quantity of progeny was calculated as biomass of sexuals of a given type eclosing per day. Regression relatedness (Grafen, 1991) was calculated as each colony queen's regression relatedness (b) with, as appropriate, new queens ($b = 0.5$), queen-produced males ($b = 1$) or worker-produced males ($b = 0.5$). Relative sex-specific reproductive value was calculated as 1 for new queens and $1/(1 + p)$ for males, where p is the population frequency of queen-produced males (Boomsma & Grafen, 1991), which equalled 0.97 and 0.70 for the IoZ and QMUL samples respectively (Table 1). Relative mating success was calculated as 1 for new queens and s for males, where $s : 1$ is the daily female : male population numerical sex ratio. The value of s was calculated from the data on number of sexuals eclosing per day (absolute dates). This calculation assumed that males are present in the mating population from days 5–25 of male adult life and, on average, mate on day 10 and new queens are present in the mating population from days 0 to 10 of queen adult life, leaving to diapause following their single mating during that time. Both these assumptions are supported by laboratory observations (Duchateau & Mariën, 1995; Beekman & Van Stratum, 1998; Tasei *et al.*, 1998). Finally, the fitness function was summed over all three classes of progeny (new queens, queen-produced males and worker-produced males) and then summed over all days over which sexuals were produced by a given colony. This function differed from similar ones (Beekman & Van Stratum, 1998; Imhoof & Schmid-Hempel, 1999; Gerloff & Schmid-Hempel, 2005) because it accounted completely for temporal variation in the population sex ratio over the period of sexual production, it employed measured rather than estimated parameters (e.g. for values of p) and it incorporated production data from the entire colony cycle. For comparison with other studies (e.g. Baer & Schmid-Hempel, 2003), we also estimated queens' lifetime reproductive success as queenright total sexual production.

To investigate predictors of queens' lifetime reproductive success, we conducted multiple regressions with worker number, switch point date and queen longevity as the independent variables. Queen longevity (as colony queen) was defined as the time in days between first worker eclosion and the queen's death. These variables were selected because: (a) they embodied logical potential predictors of sexual production (incorporating colony size, a timing variable and a measure of colony duration) and (b) none of them proved to be significantly intercorrelated, whereas other potential predictors (e.g. colony growth rate, absolute date of first worker eclosion) proved highly collinear with one or more of them. The dependent variable was either queens' inclusive fitness ($\log_{10}$ transformed in both samples to achieve a normal distribution) or queenright total sexual production ($\log_{10}$ transformed in the IoZ sample to achieve a normal distribution). Nonsignificant variables were removed (by backwards elimination) and only final models are reported.

Table 1 Summary data for IoZ and QMUL *Bombus terrestris* samples, with statistical comparisons.

	IoZ sample	QMUL sample	Comparison
Median absolute date of queen collection	11 March 2003	21 March 2004	n/a
Median absolute date of first worker eclosion	22 April 2003	2 May 2004	n/a
Number of workers produced (worker number)	82.4 ± 37.8	104.6 ± 64.8	$t_{38} = 1.55$, $P = 0.130$
Number of queens produced	4.4 ± 9.5	5.3 ± 11.3	$U_{58} = 399.5$, $P = 0.781$
Number of males produced (total)	247.4 ± 187.0	266.6 ± 157.4	$t_{56} = 0.42$, $P = 0.678$
Percentage of all males produced in queenless conditions	2.7 ± 8.8	30 ± 27 (19 colonies)	$U_{51} = 65.0$, $P = 0.000$
Total sexual production (g)	71.2 ± 57.8	75.2 ± 44.9	$t_{56} = 0.29$, $P = 0.775$
Queenright total sexual production (g)	69.8 ± 58.3	45.9 ± 34.8 (19 colonies)	$t_{49} = 1.62$, $P = 0.112$
Colony growth rate (workers/day)	1.77 ± 0.72	1.65 ± 0.84	$t_{56} = 0.55$, $P = 0.582$
Sex investment ratio (proportion of investment in females)	0.049 ± 0.131	0.049 ± 0.088	$U_{58} = 395.5$, $P = 0.730$
% resources invested in sexual production	73.2 ± 14.6	68.0 ± 15.1 (19 colonies)	$t_{49} = 1.21$, $P = 0.232$
Worker number on date of queen's death	35.8 ± 38.6	65.3 ± 39.6	$U_{51} = 153.5$, $P = 0.003$
Mean per capita fresh mass queen (mg)	799 ± 78 (129 queens)	No data	n/a
Mean per capita fresh mass male (mg)	266 ± 30 (1471 males)	No data	n/a
Switch point date (days)	17 ± 8	27 ± 13	$t_{40} = 3.11$, $P = 0.003$
Date first queen-destined egg laid (days)	33 ± 13 (9 colonies)	42 ± 8 (7 colonies)	$t_{14} = 1.78$, $P = 0.097$
Competition point date (days)	42 ± 9	No data	n/a
Queen longevity (days)	66 ± 15	82 ± 23 (19 colonies)	$t_{27} = 2.68$, $P = 0.012$

Mean ± SD, per-colony data unless otherwise stated. IoZ sample: $n = 32$ colonies; QMUL sample: $n = 26$ colonies, except where otherwise given (in parentheses). Date first queen-destined egg laid and (IoZ sample) mean per capita fresh mass of queens only from colonies producing five or more queens. Queen longevity and all dates except absolute dates are expressed as days from first worker eclosion. n/a, not applicable.

Worker reproduction under queenless conditions

In the IoZ sample, microsatellite genotyping revealed that only a small percentage of males (3.6%) was produced by workers in queenright conditions (Lopez-Vaamonde *et al.*, 2004a). The same was assumed true in the QMUL sample. Hence, for analyses in the present study, males eclosing up to and including the date of the queen's death plus 26 days were assumed to be queen produced and males eclosing after this date were assumed to be worker produced. In the IoZ sample, this yielded data on the amount of queenless worker reproduction slightly different from those in Lopez-Vaamonde *et al.* (2004a), in which a more conservative male developmental period of 31 days (the upper limit) was used.

Mass of queen- and worker-produced males

In the IoZ sample alone, the per capita fresh mass of a subset of males (those genotyped by Lopez-Vaamonde *et al.*, 2004a) was determined with an electronic balance (Oxford S1204). We compared the per capita mass of queen- and worker-produced males within colonies, the parentage of males having being established from the genetic data (Lopez-Vaamonde *et al.*, 2004a). We used GLMs designed as mixed model two-way ANOVAS, with colony as a random factor and male type as a fixed factor. To control for temporal changes in male mass (data not shown), the analyses included, within each colony, only queen-produced males that eclosed after the first worker-produced male. They also included only colonies with five or more worker-produced males each.

Definitions of variables and general statistical methods

Dates and time intervals in days are expressed relative to the date of first worker eclosion within a focal colony unless otherwise stated (in which case they are termed absolute dates). This was done to provide a standard time point from which to date events in the colony cycle. However, note that the relative dates broadly tracked absolute dates. For example, the correlation between the switch point date (relative to first worker eclosion) and the absolute switch point date was significantly positive in both samples (IoZ sample: $r_{32} = 0.58$, $P = 0.000$; QMUL sample: $r_{26} = 0.61$, $P = 0.001$). Other key variables in the analyses were defined as follows:

worker number (colony size): total number of (adult) workers produced;

colony growth rate: worker number divided by the interval in days between the eclosion of the first and last workers;

total sexual production: total fresh mass (biomass) of new queens and males produced, using colony-specific mean sexual masses for the IoZ sample and the IoZ population-wide mean sexual masses (Table 1) for the QMUL sample;

queenright total sexual production: total sexual production excluding all males produced in queenless conditions;

percentage of time invested in sexual production: interval between the eclosion of the first and last queen-produced sexual as a percentage of the interval between the eclosion of the first worker and the last queen-produced sexual;

percentage of resources invested in sexual production: queenright total sexual production as a percentage of the sum of queenright total sexual production and biomass investment in workers, taking average worker mass to be 210 mg (Holehouse *et al.*, 2003).

Sex investment ratios are expressed as the biomass proportion of sexual investment in females, where females are new queens. The female : male cost ratio used to derive sex investment ratios from numerical sex ratios was calculated as the ratio of per capita fresh masses of IoZ sexuals (=799/266 or 3.00; Table 1). Male bias in the sex ratio meant that using other forms of cost ratio (Boomsma, 1989; Beekman & Van Stratum, 1998) would barely affect estimated sex investment ratios.

All summary statistics are given as mean ± 1 SD unless otherwise stated. Data sets were checked for normality prior to parametric analyses; non-normal data were transformed to achieve normality or analysed non-parametrically. Analyses were conducted with SPSS 12.0.1 and 14 (SPSS Inc., Chicago, IL, USA). *P*-values are quoted to three decimal places.

Results

Basic demographic and production data and colony growth schedules

The 32 IoZ colonies produced 2637 workers, 140 queens and 7916 males and the 26 QMUL colonies produced 2720 workers, 138 queens and 6932 males. All colonies produced males, but only 47% (15/32) of IoZ colonies and 50% (13/26) of QMUL colonies produced queens (Table 1; Fig. 1; Supporting information).

The distributions of the switch point dates did not differ significantly from a normal distribution in either sample (Shapiro–Wilk tests, $P = 0.705$ in IoZ sample and 0.631 in QMUL sample). Therefore, there was no bimodal division into early- and late-switching colonies. Accordingly, we treated switch point as a continuous variable, and found that there was no relationship between sex investment ratio and switch point in the IoZ sample, but that there was a significant positive relationship between sex investment ratio and switch point in the QMUL sample (linear regression: IoZ sample: $F_{1,30} = 0.0$, $P = 0.999$; QMUL sample: $F_{1,24} = 9.19$, $P = 0.006$). In other words,

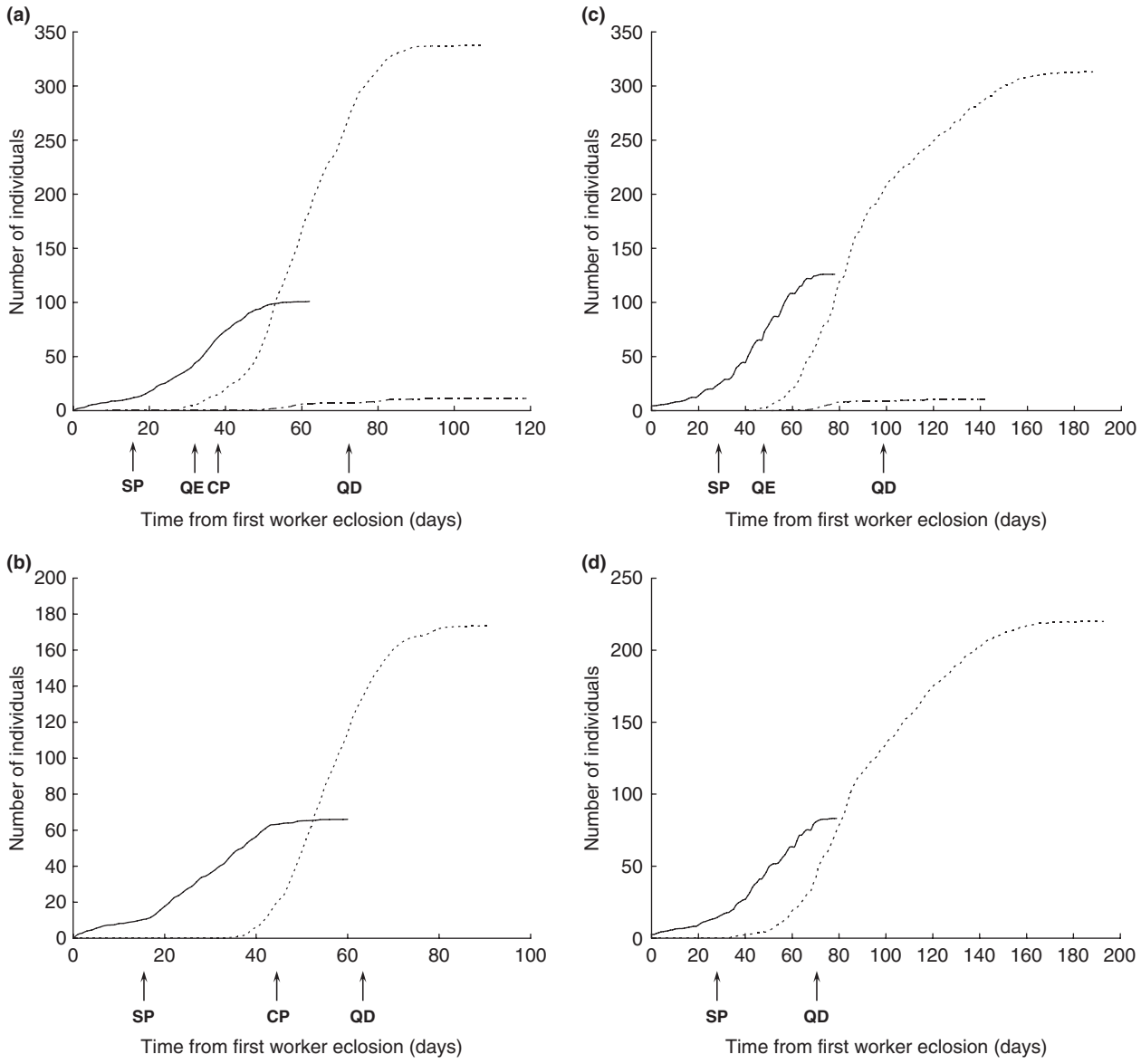

Fig. 1 Average schedules of worker and sexual production of *Bombus terrestris* colonies (i.e. per-colony average of cumulative numbers of adults eclosing per day): (a) IoZ sample, queen-producing colonies, $n = 15$; (b) IoZ sample, non-queen-producing colonies, $n = 17$; (c) QMUL sample, queen-producing colonies, $n = 13$; (d) QMUL sample, non-queen-producing colonies, $n = 13$. Solid line, workers; dotted line, males; dash-dot line, queens. Standard errors for daily mean values are in Table S2. Arrows: mean dates of competition point (CP), switch point (SP), first queen-destined egg (QE), queen's death (QD).

in the QMUL sample alone, later switching colonies produced relatively more female-biased sex ratios.

In the IoZ sample, the competition point followed the switch point in all 32 colonies, after a mean interval of 24 ± 9 days. There was a significant positive correlation between the date of the switch point and the date of the competition point (Spearman's $\rho_{32} = 0.51$, $P = 0.003$). In 10 of 15 queen-producing colonies, the competition point occurred after the estimated date at which the first queen-destined egg was laid (9 ± 5 days after), whereas in the five remaining queen-producing colonies it occurred before this date (9 ± 3 days before).

Colonies invested on average only 5% of sexual biomass in queens and this level did not differ significantly between the two samples (Table 1). Comparisons of colonies producing queens and those not producing queens within each sample showed that production of queens tended to be associated (i.e. difference significant in at least one sample, and trend same in the other) with a larger workforce, greater total and queenright sexual

production, an earlier start to colony growth (absolute date of first worker eclosion) and greater queen longevity (Table 2). Colonies producing queens tended to have later switch points (consistent with later switching colonies having more female-biased sex investment ratios in the QMUL sample), although this difference was not significant in either sample (Table 2). In the IoZ sample (data unavailable in the QMUL sample), colonies producing queens also had a significantly shorter interval between the switch and competition points (Table 2). Within the set of queen-producing colonies in both samples, the number of new queens correlated negatively with the interval from the switch point to the first queen-destined egg, although significantly so only in QMUL colonies (IoZ sample: Spearman's $\rho_{15} = -0.47$, $P = 0.076$; QMUL sample: Spearman's $\rho_{13} = -0.77$, $P = 0.002$). In other words, colonies whose queens started laying queen-destined eggs early relative to the switch point produced more queens.

Predictors of queens' lifetime reproductive success

The multiple regressions showed that queens' inclusive fitness was significantly positively associated ($P = 0.008$ and 0.043, respectively) with worker number and queen longevity in the IoZ sample (final model: $F_{2,29} = 7.2$, $r^2 = 33\%$, $P = 0.003$) and significantly positively associated with queen longevity alone in the QMUL sample ($F_{1,17} = 7.5$, $r^2 = 31\%$, $P = 0.014$). Queenright total sexual production was significantly associated (positively, negatively and positively respectively; $P = 0.000$, 0.007 and 0.000) with worker number, switch point date and queen longevity in the IoZ sample ($F_{3,28} = 16.8$, $r^2 = 64\%$, $P = 0.000$) and significantly positively associated ($P = 0.002$ and 0.000 respectively) with worker number and queen longevity in the QMUL sample ($F_{2,16} = 33.2$, $r^2 = 81\%$, $P = 0.000$). Therefore, queen longevity was consistently positively associated with queens' lifetime reproductive success (Fig. 2), with, depending on the sample and the measure of reproductive success, independent positive contributions stemming from higher worker number and an earlier switch point date. Using queens' inclusive fitness as the measure of reproductive success increased the relative fitness of queen-producing colonies (Fig. 2), as the very male-biased population sex ratio elevated the relative mating success of new queens. As queen-producing colonies tended to have longer lived queens (Table 2), this effect reinforced the positive relationship between queen longevity and reproductive success. Only a small percentage of queens' inclusive fitness (IoZ sample: 1.3 ± 6.3%, $n = 32$ colonies; QMUL sample: 5.1 ± 9.4%, $n = 19$ colonies) consisted of indirect fitness returns from males produced by queenless workers. This was because of the male-biased population sex ratio depressing the fitness returns from males, and because males produced by queenless workers tended to be produced late, when queens with which they would have been able to mate were no longer being produced. Therefore, most of the fitness of queens stemmed from their own sexual productivity.

Worker number significantly correlated with colony growth rate (IoZ sample: $r_{32} = 0.93$, $P = 0.000$; QMUL sample: $r_{26} = 0.97$, $P = 0.000$), indicating that faster growing colonies attained a greater final size and did not cease growing at a lower final size. Queen

Table 2 Comparison of colonies producing queens and colonies not producing queens in *Bombus terrestris*.

	Colonies producing queens	Colonies not producing queens	Comparison
IoZ sample			
Worker number	101 ± 39	66 ± 29	$t_{30} = 2.90$, $P = 0.007$
Number of males produced (queenright conditions)	327.0 ± 222.2	167.5 ± 114.5	$t_{20} = 2.50$, $P = 0.021$
Total sexual production (g)	103.4 ± 65.5	42.7 ± 29.7	$t_{19} = 3.30$, $P = 0.004$
Queenright total sexual production (g)	102.2 ± 65.5	41.1 ± 29.2	$t_{19} = 3.29$, $P = 0.004$
Absolute date of first worker eclosion	17 April ± 8 days	25 April ± 10 days	$t_{30} = 2.60$, $P = 0.014$
Switch point date (days)	19 ± 7	16 ± 10	$t_{30} = 0.81$, $P = 0.424$
Competition point date (days)	39 ± 7	44 ± 11	$t_{30} = 1.55$, $P = 0.130$
Interval between switch and competition points (days)	20 ± 7	28 ± 9	$t_{30} = 2.67$, $P = 0.012$
Queen longevity (days)	70 ± 17	62 ± 13	$t_{30} = 1.57$, $P = 0.126$
QMUL sample			
Worker number	126 ± 57	83 ± 67	$t_{24} = 1.75$, $P = 0.093$
Number of males produced (queenright conditions)	238.9 ± 133.5 (7 colonies)	123.1 ± 109.5 (12 colonies)	$t_{17} = 2.05$, $P = 0.056$
Total sexual production (g)	91.8 ± 37.6	58.5 ± 46.7	$t_{24} = 2.00$, $P = 0.057$
Queenright total sexual production (g)	68.6 ± 33.6 (7 colonies)	32.7 ± 29.1 (12 colonies)	$t_{17} = 2.45$, $P = 0.026$
Absolute date of first worker eclosion	4 May ± 11 days	15 May ± 18 days	$t_{20} = 1.76$, $P = 0.094$
Switch point date (days)	30 ± 12	24 ± 15	$t_{24} = 1.09$, $P = 0.284$
Queen longevity (days)	98 ± 14 (7 colonies)	72 ± 23 (12 colonies)	$t_{17} = 2.68$, $P = 0.016$

Mean ± SD, per-colony data. IoZ sample: $n = 15$ and 17 colonies respectively; QMUL sample: $n = 13$ and 13 colonies, respectively, except where otherwise given (in parentheses). Queen longevity and all dates except absolute dates are expressed as days from first worker eclosion. In the QMUL sample, there were no data on competition point date.

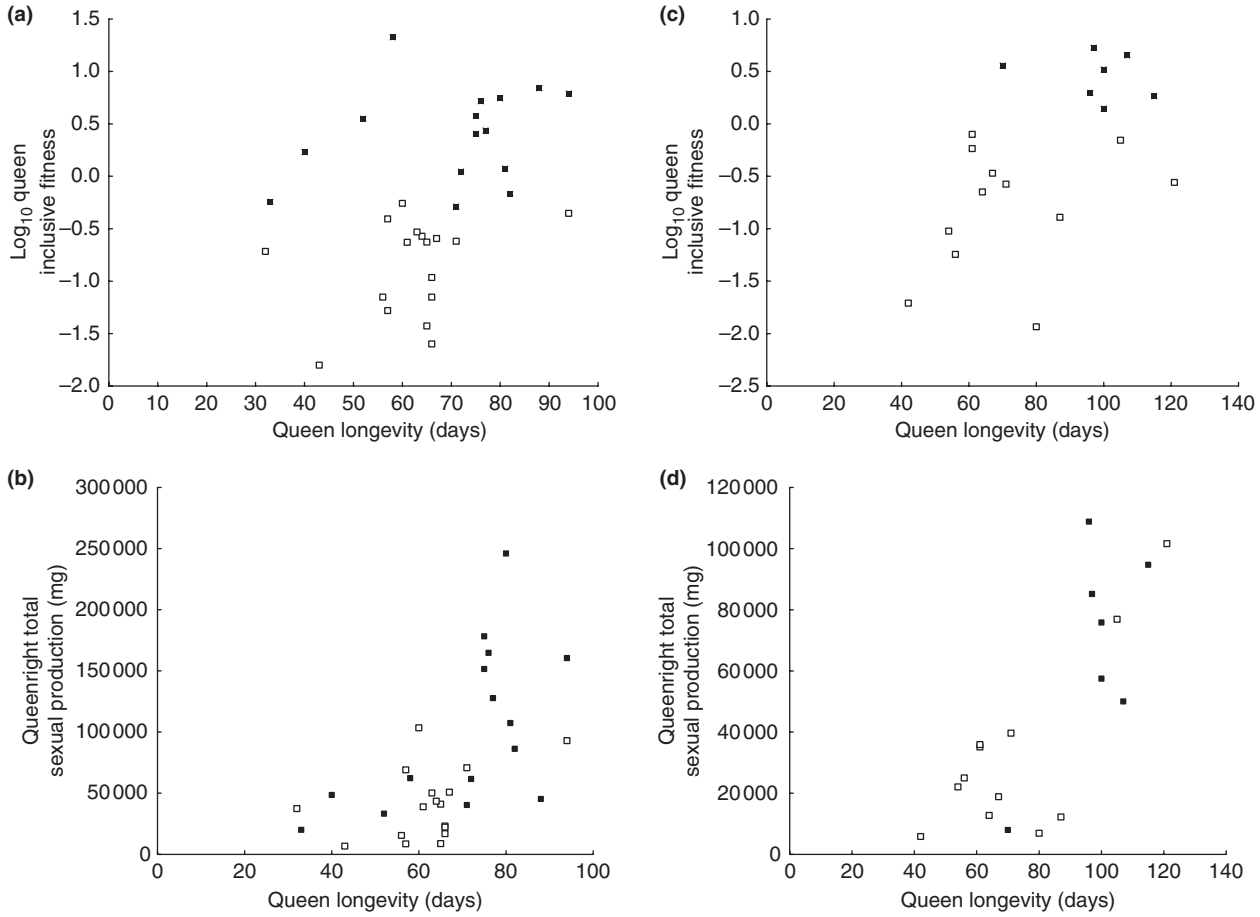

Fig. 2 Relationship between lifetime reproductive success and queen longevity (days from first worker eclosion to queen's death) in *Bombus terrestris* colonies (bivariate plots for illustration only): (a) IoZ sample, reproductive success measured as $\log_{10}$ queen inclusive fitness, $n = 32$; (b) IoZ sample, reproductive success measured as queenright total sexual production (mg), $n = 32$; (c) QMUL sample, reproductive success measured as $\log_{10}$ queen inclusive fitness, $n = 19$; (d) QMUL sample, reproductive success measured as queenright total sexual production (mg), $n = 19$. Filled squares, queen-producing colonies; unfilled squares, colonies not producing queens.

longevity significantly correlated with percentage of time (IoZ sample: $r_{32} = 0.50$, $P = 0.004$; QMUL sample: Spearman's $\rho_{19} = 0.62$, $P = 0.005$) and resources (IoZ sample: $r_{32} = 0.63$, $P = 0.000$; QMUL sample: $r_{19} = 0.60$, $P = 0.006$) invested in sexual production. These findings suggested that all colonies invested similar time and resources in worker production, but that more reproductively successful colonies were thereafter able to invest larger amounts in reproduction in proportion to their queens' longevity. However, there was no significant association between queen longevity and either relative or absolute date of the switch point (linear regression: IoZ sample: relative switch point date: $F_{1,30} = 0.2$, $P = 0.656$; absolute switch point date: $F_{1,30} = 1.87$, $P = 0.182$; QMUL sample: relative switch point date: $F_{1,17} = 0.1$, $P = 0.722$; absolute switch point date: $F_{1,17} = 2.17$, $P = 0.159$). This suggested that queens with a lower expectation of life as a colony queen did not switch to male production relatively early.

Worker reproduction under queenless conditions

The frequency of colonies producing males in queenless conditions was significantly lower in the IoZ sample than in the QMUL sample (IoZ sample: 6 of 32 colonies; QMUL sample: 17 of 19 colonies; corrected $\chi^2_1 = 21.3$, $P = 0.000$). In addition, QMUL colonies had a significantly greater percentage of males produced by workers in queenless conditions (Table 1). (Excluding drifter worker reproduction in the IoZ sample from these comparisons would exaggerate the differences detected, as drifter workers produced about 60% of worker-produced males in queenless conditions; Lopez-Vaamonde *et al.*, 2004a.) In the IoZ sample, colonies in which workers produced males under queenless condi-

tions exhibited a significantly shorter interval between the switch point and the queen's death than those in which workers did not produce males under queenless conditions (28 ± 14 vs. 53 ± 14 days respectively; $t = 3.91$, d.f. = 30, $P = 0.000$). Similarly, in the QMUL sample, there was a significant negative relationship between the percentage of males produced by workers under queenless conditions and the interval between the switch point and the queen's death (linear regression: $F_{1,17} = 20.2$, $P = 0.000$; Fig. 3). Therefore, in both samples, the sooner the queen died relative to the switch point, the greater the direct reproductive success achieved by workers in queenless conditions.

Mass of queen- and worker-produced males

In the IoZ sample, there was a significant positive correlation across colonies between the mean per capita mass of queen-produced males and the number reared ($r_{32} = 0.35$, $P = 0.049$). Six colonies contained five or more worker-produced males each. In the first GLM, to increase sample sizes, we pooled males produced by resident workers and males produced by drifter workers. Queen- and worker-produced males did not differ significantly in per capita mass (global means = 264 ± 63 and 265 ± 56 mg, $n = 80$ and 57 males, respectively, with the 57 worker-produced males comprising 18 produced by resident and 39 produced by drifter workers; $F_{1,5} = 0.01$, $P = 0.920$). There was a significant effect of colony on male mass ($F_{5,125} = 6.58$, $P = 0.000$) but no significant interaction between male type and effect of colony ($F_{5,125} = 1.89$, $P = 0.100$). A second GLM compared per capita mass of queen-produced males and males produced by drifter workers alone ($n = 54$ and 37 males, respectively, from four of the six colonies). Again, queen- and worker-produced males did not differ significantly in per capita mass (effect of male type: $F_{1,3} = 3.37$, $P = 0.160$; effect of colony: $F_{3,83} = 4.83$, $P = 0.004$; interaction: $F_{3,83} = 0.63$, $P = 0.598$). Sample sizes were too low for the complementary analysis comparing masses of queen-produced males and males produced by resident workers alone. Overall, these results showed that male mass was determined by colony identity, probably through the influence of colony productivity, irrespective of male parentage. This confirmed the assumption used in our function for queens' inclusive fitness that male parentage does not affect males' intrinsic quality.

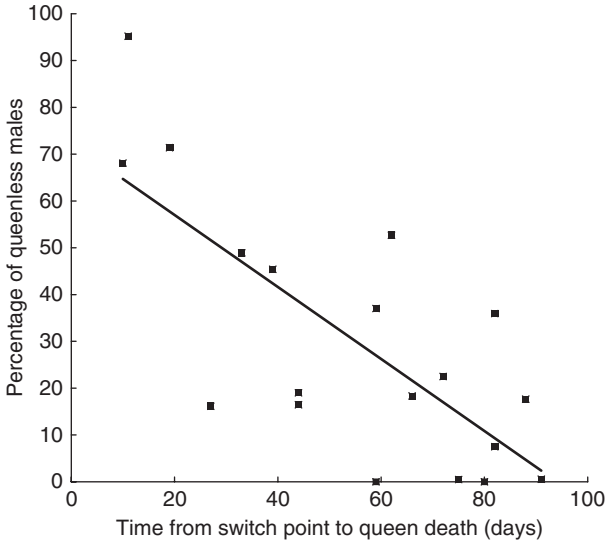

Fig. 3 Relationship between percentage of males produced by workers under queenless conditions and the interval from the switch point to the date of the queen's death in *Bombus terrestris* colonies (QMUL sample, $n = 19$ colonies). Regression line is $y = -0.77x + 72.4$ ($F_{1,17} = 20.2$, $P < 0.000$).

Discussion

Relationship between queens' lifetime reproductive success and longevity

The goals of the present study were to establish the relationship between queens' lifetime reproductive success and their longevity in a social insect and to investigate other factors that potentially affect this relationship, including within-colony conflict. In two samples of colonies from a population of the annual bumblebee, *B. terrestris audax*, we found a consistent positive relationship between queens' lifetime reproductive success and longevity. This relationship has not previously been demonstrated in any social insect. However, in agreement with this finding, positive correlations have been reported between colony age and size in *B. lucorum* (Müller & Schmid-Hempel, 1992a) and between sexual production and the period of sexual eclosions in *B. t. terrestris* (Imhoof & Schmid-Hempel, 1999). Our results therefore show that *B. terrestris* queens do not engage in rapid, early sexual production (through a rapid initial rate of worker production) at the expense of their later survival. In fact, higher colony growth rates were associated with greater colony sizes and hence, indirectly, with greater queen longevity. Overall, queens achieved increasing lifetime reproductive success in proportion to the amount of time that they remained alive as colony queens because longer lived queens headed colonies investing relatively more time and resources in sexual production.

Our data also shed light on the potential role of conflict in determining the relationship between queens' lifetime reproductive success and longevity. In both samples, relatively earlier queen's death was significantly associated with greater levels of worker reproduction in queenless conditions. [Although the extent of queenless worker reproduction in the QMUL colonies was likely to have been elevated because their confinement allowed relatively more workers to outlive the queen (Table 1),

the data from the IoZ sample show that queenless worker reproduction occurs in free-foraging colonies too.] In principle, worker matricide should occur in *B. terrestris* when the fitness benefit to workers from matricide followed by worker male production exceeds the fitness cost to them of not rearing the queen-produced sexual siblings they would have reared had the queen remained alive (Bourke, 1994). Our data show that, on the one hand, workers obtain increasing direct fitness benefits from queenless worker reproduction if their queen dies relatively early (Fig. 3) and, on the other hand, workers obtain increasing indirect fitness benefits from the greater sexual productivity of longer lived queens (Fig. 2). In effect, these relationships set the parameters of the benefit–cost ratio for worker matricide. In the present study, we do not know if queens died through matricide or intrinsic causes (although, consistent with matricide, in one colony in the IoZ sample, the queen's corpse was found to be mangled). A detailed test of whether workers commit matricide at the appropriate benefit–cost ratio therefore awaits further study. Nonetheless, the overall positive relationship between queens' lifetime reproductive success and longevity shows that, even if matricide occurs frequently, queens do not apparently compensate in such a way as to alter the directionality of this relationship.

There are several possible evolutionary causes of a positive relationship between queens' lifetime reproductive success and longevity in *B. terrestris*. One is that, like parental care in the nonsocial semelparous Hymenoptera that are likely to resemble the ancestors of annual social bees (Bosch & Vicens, 2006), sociality permits concurrent increases in these two life-history traits. Specifically, workers in social insects simultaneously provide aid to queens, which is likely to increase queens' reproductive success, and protect them against sources of extrinsic mortality (Keller & Genoud, 1997), which is likely to increase queens' longevity. In addition, as in their nonsocial semelparous ancestors, annual social insects enjoy a life history providing a relatively predictable period available for colony growth and reproduction (Bourke, 1994; Martin, 1995; Beekman *et al.*, 1998), facilitating selection on reproductive females to maximize the amount of time spent producing progeny within this period. Overall, however, establishing the validity of these suppositions awaits far more extensive life-history information from both nonsocial and social Hymenoptera.

Future studies should also extend the study of life history over the entire colony cycle to fully natural conditions and (as far as possible) to perennial social insects, and should experimentally investigate the proximate causes of life-history patterns. However, note that the positive association between queens' lifetime reproductive success and longevity found in the present study did not arise through our colonies being kept in conditions of partial (IoZ sample) or complete (QMUL sample) confinement. First, the two samples differed strongly in the extent to which workers and nests were exposed to sources of extrinsic mortality. For example, seven IoZ colonies were eventually destroyed by larvae of wax moth (*Aphomia sociella*), which must have developed from eggs laid by adult moths entering the nest-boxes from the exterior, whereas QMUL workers and colonies were not exposed to external predation. Nonetheless, despite this difference, the samples were remarkably similar in their growth schedules and patterns of within-sample demographic correlation (Table 1; Figs 1 and 2), showing that extrinsic mortality had little effect on the life-history decisions of colonies. Second, previous studies have demonstrated that even large differences in external worker mortality fail to have substantial effects on colony growth schedules in *B. terrestris* and its relatives (Müller & Schmid-Hempel, 1992b; Baer & Schmid-Hempel, 2003). This suggests that reduced external mortality in our samples is unlikely to have substantially affected the life-history decisions of colonies. Instead, the strong resemblance between our samples is consistent with previous demographic (Archer, 1981; Martin, 1991, 1995; Müller *et al.*, 1992; Bourke, 1997; Duchateau *et al.*, 2004) and experimental (Beekman & Van Stratum, 2000; Cnaani *et al.*, 2000; Lopez-Vaamonde *et al.*, 2004b; Alaux *et al.*, 2005; Liebig *et al.*, 2005) data suggesting that traits of colony queens exert a large influence on colony life history in *Bombus* species in particular and annual social insects in general. Variation evident within our samples (Fig. 2) in queen longevity (and hence in queens' lifetime reproductive success) is therefore likely to have stemmed from variation in queen quality (e.g. conceivably, nutritional status and fat content), although the most influential queen traits in this context remain unknown.

Secondary correlates of queens' lifetime reproductive success were worker number and, in the IoZ sample, an early switch to male production. The first of these was expected, given that workers rear the sexuals, and confirms previous results from both annual and perennial social insects (e.g. Schmid-Hempel *et al.*, 1993; Martin, 1995), including bumblebees (Bourke, 1997). The positive effect of an earlier switch point (in the IoZ sample) was most likely linked to the more productive colonies being those with a longer relative period of sexual production. Our data did not support the hypothesis of Duchateau *et al.* (2004) that early switching is associated with reduced queen life expectancy, perhaps because our study population, unlike that of Duchateau *et al.* (2004), did not exhibit bimodality in the switch point.

We estimated queens' inclusive fitness assuming that queen- and worker-produced males did not differ in intrinsic quality. Because we found no significant differences within colonies between the per capita masses of queen- and worker-produced males, even when worker-produced males derived from unrelated reproductive

drifter workers, our data suggest that this assumption was justified. Male mass correlated positively with number of males produced and, so, instead of being determined by caste of parentage, was presumably determined by the rearing environment in each colony. Gerloff & Schmid-Hempel (2005) found *B. terrestris* male size to be significantly negatively correlated with number produced, suggesting a dependence on rearing environment of opposite sign. Studies comparing traits of queen- and worker-produced males in *Acromyrmex* ants also found no systematic difference in male body size between the two male types (Camargo *et al.*, 2005; Dijkstra & Boomsma, 2007).

Other life-history issues in *Bombus terrestris*

The findings of the present study confirm that the *B. terrestris* colony cycle is characterized by a period of exclusively worker production followed by a period of mixed worker and sexual production followed by a period of exclusively sexual production (Müller *et al.*, 1992) (Fig. 1). Therefore, on a coarse scale, colonies exhibited the 'bang-bang' strategy (sequence involving investment in growth followed by investment in reproduction, separated by a sharp transition) predicted by life-history models developed for semelparous organisms or groups such as monocarpic plants and annual insect societies (Macevicz & Oster, 1976; Metcalf *et al.*, 2003; Mitesser *et al.*, 2007). Mechanistically, this is achieved in *B. terrestris* by a relatively sharp switch point (Duchateau & Velthuis, 1988; Duchateau *et al.*, 2004). Clearly, however, in our samples there was some degree of gradation in the transition from worker to sexual production (Fig. 1). There has been extensive discussion in the literature of whether such 'graded control' of reproduction, which is commonly observed in annual social insects, is consistent with 'bang-bang' model predictions or whether it can be ascribed to other factors (Macevicz & Oster, 1976; Greene, 1984; Martin, 1991; Müller *et al.*, 1992; Beekman *et al.*, 1998; Mitesser *et al.*, 2007). However, existing models do not define the degree of gradation that would constitute significant departure from a 'bang-bang' pattern. In the absence of more sophisticated models with this property, in our view it is therefore not possible quantitatively to test the 'bang-bang' prediction at present.

Our data also confirmed, to varying degrees, several other patterns demonstrated in previous studies of *B. terrestris* or other *Bombus* species. For example, a significant positive correlation between the date of the switch point and the date of the competition point in *B. terrestris* was found by Duchateau *et al.* (2004). These authors also reported that the competition point occurs 12 days after the first queen-destined egg is laid. In our study a majority (10 of 15 queen-producing colonies in the IoZ sample) conformed to this pattern (competition point occurred a mean 9 days after the first queen-destined egg), whereas, for unknown reasons, a minority of colonies (remaining five IoZ queen-producing colonies) clearly contradicted it (competition point occurred a mean 9 days before the first queen-destined egg). Our results also confirmed highly male-biased population sex investment ratios in *B. terrestris* as found in previous studies of *B. t. audax* (Ings *et al.*, 2006), *B. t. terrestris* (Shykoff & Schmid-Hempel, 1991) and other *Bombus* species (Bourke, 1997; Pelletier & McNeil, 2003). Such male-biased sex investment appears associated with protandry (Bourke, 1997), which we also found (Fig. 1). However, our population differed from the Dutch *B. t. terrestris* population, which exhibits an unbiased population sex investment ratio and colony-level sex ratio bimodality associated with a bimodal switch point (Duchateau *et al.*, 2004). We found instead that switch point bimodality was absent, but that, at least in the QMUL sample, later switching colonies still tended to produce a relatively more female-biased sex investment ratio. The differences between the Dutch and UK populations suggest substantial interpopulation or subspecific variation in this suite of traits. Within our samples, colonies producing queens tended to be larger and more productive than those not producing queens (Table 2). This again matched a general pattern in both bumblebees (Bourke, 1997; Pelletier & McNeil, 2003; Gerloff & Schmid-Hempel, 2005) and vespine wasps (Archer, 1981).

A novel correlate of queen production was the date of laying of the first queen-destined egg; the sooner this date relative to the switch point, the greater the number of queens produced. This relationship could have driven the negative association found by both Duchateau *et al.* (2004) and ourselves between queen production and the interval between the switch and competition points. In *B. terrestris*, evidence exists that early-instar female larvae undergo determination as queens when the colony queen ceases production of a putative pheromone, with this event triggering the competition point several days later (Bloch, 1999; Cnaani *et al.*, 2000; Bourke & Ratnieks, 2001; Duchateau *et al.*, 2004; Alaux *et al.*, 2006). This would generate the delay between the first queen-destined egg and the competition point found in the study of Duchateau *et al.* (2004) and in the majority of our IoZ colonies. Therefore, at the proximate level, colonies producing fewer queens could be those in which the queen ceases pheromone production too late following the switch point, when fewer diploid eggs remain for development as queens.

Conclusions

We have shown for the first time that, in an annual social insect, queens' lifetime reproductive success is positively associated with queens' longevity. The proximate reason for this is that longer lived queens invest a disproportionately greater relative amount of time and resources in

reproduction. A positive relationship between lifetime reproductive success and longevity does not occur in many nonsocial insects, whether semelparous or iteroparous, in which females exhibit a negative relationship (high female reproductive success comes at the cost of reduced longevity). However, insofar as the scant data show, it does occur in semelparous nonsocial Hymenoptera with parental care. This suggests that annual social Hymenoptera may have inherited a positive relationship of lifetime reproductive success and longevity from their nonsocial ancestors, with factors associated with sociality, such as queens being buffered by their workforce against extrinsic mortality, having acted to maintain it. Conflict between the queens and workers induced by sociality does not appear to have altered this relationship. However, this and several other aspects of life-history strategy in social insects require further theoretical and empirical investigation.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Demographic, temporal and production data for individual colonies.
Table S2 Cumulative growth and sexual production of colonies from date of first worker eclosion.

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